BIO2013P1

NON-CBI SUBMISSION

Form Approved. O.M.B. Nos. 2070-0012 and 2070-0038

EPA Biotech Form

Biotech Form Report Number	ALGE131227773024113	Mark (X) if anything is CBI ☐

I. SUBMITTER IDENTIFICATION INFORMATION — CBI ☐

First Name	Patrick		Last Name	Ahlm	
Position	Assistant Director, Government &		Company	Algenol Biofuels Inc.	
Mailing Address (Number & Street)	16121 Lee Road				
City	Fort Myers	State	FL	Postal Code	33912
e-mail	pat.ahlm@algenol.com		Telephone (include area code)		952-567-0943

Ia. JOINT SUBMITTER -- If you are submitting this notice as part of a joint submission, mark (X) ☐ CBI ☐

First Name			Last Name		
Position			Company		
Mailing Address (Number & Street)					
City		State		Postal Code	
e-mail			Telephone (include area code)		

II. TECHNICAL CONTACT IDENTIFICATION INFORMATION — CBI ☐

First Name	Paul		Last Name	Roessler	
Position	Chief Scientific Officer		Company	Algenol Biofuels Inc.	
Mailing Address (Number & Street)	16121 Lee Road				
City	Fort Myers	State	FL	Postal Code	33912
e-mail	paul.roessler@algenol.com		Telephone (include area code)		239-498-2000

III. TYPE OF SUBMISSION (Check One)

- ☒ MCAN (Microbial Commercial Activity Notice)
- ☐ TERA (TSCA Experimental Release Application)
- ☐ Tier I Exemption
- ☐ Tier II Exemption
- ☐ Biotech TME (Test Market Exemption)

IV. TEXT / COMMENTS — CBI ☐

Please note that 6 files are being submitted. The first three have the following file names: CONFIDENTIAL Microbial Commercial Activity Notice (12 20 13) final; CONFIDENTIAL Microbial Commercial Activity Notice (12 20 13) final.2; and CONFIDENTIAL Microbial Commercial Activity Notice (12 20 13) final.3. The files represent the version of the MCAN that does include confidential business information, due to a formatting issue they were saved as separate files. However, together they represent the complete MCAN along with the other attachments noted in the document.

The fourth file is identified as CONFIDENTIAL Microbial Commercial Activity Notice (12 20 13) final - Sanitized. This file represents the MCAN with confidential business information redacted and is intended to be published by EPA when appropriate.

The fifth file is a publicly available document from the Center for Disease Control submitted as reference and back up material.

The sixth file is a GenBank file that includes genome sequencing information for the organism identified in the MCAN. As noted in the MCAN, this file is submitted as confidential business information and should not be published.

Please contact me, Patrick Ahlm, with any questions at either 239.498.2000 or 952.567.0943.

Thank you.

LIST OF ATTACHMENTS

#	Attachment Name	Attachment Filename	Number of Pages	Associated Section Number	CBI
001	Commercial Activity Notice (12 20 13) final - Sanitized	CONFIDENTIAL Microbial Commercial Activity Notice (12	88		
002	Microbial Commercial Activity Notice (12 20 13) final.2 - Sanitized	CONFIDENTIAL Microbial Commercial Activity Notice (12	2		
003	Microbial Commercial Activity Notice (12 20 13) final.3 - Sanitized	CONFIDENTIAL Microbial Commercial Activity Notice (12	15		
004	Center for Disease Control - Facts About Cyanobacteria	CDC Facts About Cyanobacteria 12.20.13.pdf	7		
005	GenBank File Sanitized Notice	GenBank File Sanitized Notice.docx	0		

Mark (X) this box if the data continues on the next page. ☐

ATTACHMENT HEADER SHEET

Attachment Number 001

Attachment Name

Commercial Activity Notice (12 20 13) final - Sanitized

Associated PMN Section Number

N/A

Does not contain CBI

Report Number

ALGE131227773024113

ATTACHMENT HEADER SHEET

Attachment Number 002

Attachment Name

Microbial Commercial Activity Notice (12 20 13) final.2 - Sanitized

Associated PMN Section Number

N/A

Does not contain CBI

Report Number

ALGE131227773024113

ATTACHMENT HEADER SHEET

Attachment Number 003

Attachment Name

Microbial Commercial Activity Notice (12 20 13) final.3 - Sanitized

Associated PMN Section Number

N/A

Does not contain CBI

Report Number

ALGE131227773024113

ATTACHMENT HEADER SHEET

Attachment Number 004

Attachment Name

Center for Disease Control - Facts About Cyanobacteria

Associated PMN Section Number

N/A

Does not contain CBI

Report Number

ALGE131227773024113

ATTACHMENT HEADER SHEET

Attachment Number 005

Attachment Name

GenBank File Sanitized Notice

Associated PMN Section Number

N/A

Does not contain CBI

Report Number

ALGE131227773024113

ALGENOL
BIOFUELS
Harnessing the sun to fuel the world®

Microbial Commercial Activity Notice

40 C.F.R. § 725

Date of Submission: December 20,2013

Filed with: US Environmental Protection Agency
Office of Pollution Prevention and Toxics
Chemical Control Division
New Chemicals Notice Management Branch

Submitter: Algenol Biofuels Inc.
16121 Lee Road
Fort Myers, FL 33912

Submitted to: Central Data Exchange

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3 Substantiation of Confidential Business Information

December 20, 2013

Maria Doa, Director
Chemical Control Division
Office of Pollution Prevention and Toxics
Chemical Safety & Pollution Prevention
US Environmental Protection Agency

RE: Justification for Claims of Confidential Business Information

Dear Director Doa:

This letter contains justifications for the claims of confidential business information (CBI claims) contained in the Microbial Commercial Activity Notice (MCAN) submitted by Algenol Biofuels Inc. (Algenol) on December 20, 2013.

A. Information Claimed as CBI.

Algenol claims that the following information comprises Confidential Business Information and should not be shared publicly. These elements have been redacted from the "sanitized" version of Algenol's MCAN for use on EPA's Public Website:

- Identification of the cyanobacteria strain used in Algenol's technology;
- The location in the continental United States from which the cyanobacteria strain was obtained;
- Genetic sequences relating to the cyanobacteria;
- Nature, materials, and methods of genetic enhancements relating to the cyanobacteria strain
- Algenol's processes for cultivating the cyanobacteria strain and controlling contamination
- Algenol's next-generation photobioreactor systems and processes for inoculating and cleaning photobioreactors
- Algenol's systems and processes for managing photobioreactor operations, including gas and liquid flow, and for mixing cultures of the cyanobacteria strain cultured in the photobioreactors
- Algenol's product extraction and recovery systems and other downstream processes.

B. Support Information for Confidentiality Claims.

In accordance with the guidance provided by EPA's website relating to information claimed as confidential in (http://www.epa.gov/oppt/tsca8e/pubs/confidentialbusinessinformation.html) Algenol offers the following information in support of its CBI claims.

1. **Is your company asserting this confidential business information (CBI) claim on its own behalf? If the answer is no, please provide company name, address and telephone number of entity asserting claim.**

Algenol submits this CBI claim on its own behalf.

2. **For what period do you assert your claim(s) of confidentiality? If the claim is to extend until a certain event or point in time, please indicate that event or time period. Explain why such information should remain confidential until such point.**

Algenol has filed and will file patent applications that disclose and claim the following inventions, among others:

- The identity of the cyanobacteria strain used in Algenol's technology and the genetic sequences and nature, materials, and methods of genetic enhancements relating to the cyanobacteria strain
- Algenol's processes for cultivating the cyanobacteria strain and controlling contamination
- Algenol's next-generation photobioreactor systems and processes for inoculating and cleaning photobioreactors
- Algenol's systems and processes for managing photobioreactor operations, including gas and liquid flow, and for mixing cultures of the cyanobacteria strain cultured in the photobioreactors
- Algenol's product extraction and recovery systems and processes

The information disclosed in Algenol's patent applications may be published by the U.S. Patent & Trademark Office and other international and national patent agencies. Because the dates of such future publications are not known, Algenol's Confidential Business Information relating to these inventions should not be shared publicly prior to January 1, 2017.

3. **Has the information that you are claiming as confidential been disclosed to any other governmental agency, or to this Agency at any other time? Identify the Agency to which the information was disclosed and provide the date and circumstances of the same. Was the disclosure accompanied by a claim of confidentiality? If yes, attach a copy of said document reflecting the confidentiality agreement.**

Algenol has disclosed portions of CBI to several governmental agencies in an effort to develop a proactive and transparent regulatory process and active patent filing and prosecution in the US and worldwide. In each case, active measures were used to protect information. We did not disclose information without assurances of confidentiality.

The following information claimed as CBI has been disclosed to Department of Agriculture and Consumer Services in Florida. Those disclosures took place continuously in 2007-present in connection with obtaining state-level regulatory approvals related to Algenol's operations.

The following information claimed as CBI has been disclosed to the United States Patent and Trademark Office as part of a patent application. Those disclosures took place on or about 2007-present. Please see the response to question 2 for the time period for which the CBI claim is asserted as to that information.

Starting April 2012 until December 2012, the US Internal Revenue Service (IRS) conducted an audit of the Company. CBI was disseminated to the IRS during that time related to the Company's intellectual property around organisms, molecular biology and photobioreactors. Substantial discussion around IRS privacy law and disclosure were discussed with IRS agents prior to disclosure of CBI.

Algenol has disclosed to the U.S. Department of Energy certain Confidential Business Information relating to inventions created in conjunction with DOE Assistance Agreement Award No. DE-EE0002867 ("Subject Inventions"). Algenol has filed and will file patent applications that disclose and claim such Subject Inventions. The information disclosed in Algenol's patent applications may be published by the U.S. Patent & Trademark Office and other international and national patent agencies. Because the dates of such future publications are not known, Algenol's Confidential Business Information relating to such Subject Inventions should not be shared publicly prior to January 1, 2017.

4. Briefly describe any physical or procedural restrictions within your company relating to the use and storage of the information you are claiming CBI.

Algenol internal policies and procedures amply provide physical and procedural restrictions relating to the use and storage of the information claimed as CBI, identified above. Those restrictions derive from a number of sources, described below.

a. Algenol's Intellectual Property Policy. Algenol's intellectual property policy applies to prospective, current, and departing Algenol employees. Under that policy, employees:

- Agree in writing (i) not to disclose Algenol's CBI, and (ii) that during their employment and for a reasonable period following their employment they will forego participating in activities similar to those they pursued at Algenol;
- Are provided with training programs and presentations informing employees about CBI and its value and protection;
- Who are departing Algenol are provided with information regarding their obligations as to Algenol's CBI, including a departure or exit letter;
- Are required to (i) recognize and clearly mark CBI at the time of creation; (ii) restrict access to and distribution of CBI to those who require it and will keep it confidential; (iii) require advance agreements from third parties who require access to CBI; and (iv) properly and securely store CIB.
- Face disciplinary action up to an including termination for failure to follow Algenol's intellectual property policy.

b. Algenol's Employee Handbook. Algenol's employee handbook, distributed and acknowledge by all employees, also addresses issues related to the handling and safeguarding of CBI. Specifically, Algenol employees:

- Agree not to use any CBI except to perform work for the company;
- Agree not to discuss or share any CBI information with anyone else in the company except those who are authorized and have a need to know;
- Acknowledge that their duty of confidentiality continues after employment with Algenol ceases;
- Expressly acknowledge the potential for personal liability for loss or damage attributable to violation of Algenol's CBI policy.

c. Algenol's Data Security Protocol. Algenol maintains a carefully crafted data security protocol to protect CBI information, including:

- Erecting firewalls and implementing password protection to protect Algenol's servers, folders, and sites;
- Requiring two-factor authentication for outside access to Algenol's servers;
- Requiring complex usernames and passwords; and
- Encrypting electronic data, among other measures.

5. If anyone outside your company has access to any of the information claimed CBI, are they restricted by confidentiality agreement(s). If so, explain the content of the agreement(s).

Every party that either has access to confidential and proprietary information or has access to our facilities is required to enter into a Non-Disclosure Agreement (NDA). Employees, consultants and employment candidates are required to enter into NDAs as well. Third parties with a specific "need to know" are permitted to access Algenol CBI so long as they agree to terms of a Non-Disclosure Agreement (NDA). A sample NDA used by Algenol with such third parties is attached. See also the responses to Questions 3 and 4.

6. Does the information claimed as confidential appear or is it referred to in any of the following:

a. Advertising or promotional material for the chemical substance or the resulting and product.

RESPONSE: No.

b. Material safety data sheets or other similar materials (such as technical data sheets) for the substance or resulting end product (include copies of this information as it appears when accompanying the substance and/or product at the time of transfer or sale).

RESPONSE: No.

c. Professional or trade publications.

RESPONSE: No.

d. Any other media or publications available to the public or to your competitors.

RESPONSE: No.

7. Has EPA, another federal agency, or court made any confidentiality determination regarding information associated with this substance? If so, provide copies of such determinations?

No formal determinations; however, information provided to IRS and DOE were kept confidentially.

8. Describe the substantial harmful effects that would result to your competitive position if the CBI information is made available to the public? In your answer, explain the causal relationship between disclosure and any resulting substantial harmful effects. Consider in your answer such constraints as capital and marketing cost, specialized technical expertise, or unusual processes and your competitors access to your customers. Address each piece of information claimed CBI separately.

Algenol is a start-up company pioneering a completely novel and proprietary technology, with first-of-its kind equipment and processing. To be responsive to the MCAN regulations, the Company has disclosed its commercial strain and how to make it, as well as significant aspects of its upstream and downstream processing based upon pre-conferences with EPA personnel. Disclosure of this information would result in adverse, if not devastating consequences to the Company.

Its sources of capital have been investors who view Algenol's technology and IP protections as valuable. In fact, Algenol was awarded the DOE Grant on the basis of its novel technology and intellectual property. Algenol would either cease to be in existence or would lose significant value if any of the CBI information were to be disclosed prior to the issuance of a patent. In such a case, the likelihood of mass reductions in its labor force would increase.

9. Has the substance been patented in the U.S. or elsewhere? Is a patent for the substance currently pending?

Yes. Algenol owns or licenses the following patents:

COUNTRY	PATENT NUMBER	TITLE	GRANT DATE
Australia	779434	Genetically Modified Cyanobacteria for the Production of Ethanol	May 26, 2005
Australia	2007207635	Methods and Compositions for Ethanol Producing Cyanobacteria	May 2, 2013
Australia	2007313669	Closed Photobioreactor System for Production of Ethanol	Jun 25, 2013
Canada	2,281,953	Genetically Modified Cyanobacteria for the Production of Ethanol	Nov 27, 2012
European Patent Office	EP0963439	Genetically Modified Cyanobacteria for the Production of Ethanol	Apr 11, 2007
European Patent Office	1979481	Methods and Compositions for Ethanol Producing Cyanobacteria	Mar 2, 2011
France	EP0963439	Genetically Modified Cyanobacteria for the Production of Ethanol	Apr 11, 2007
France	1979481	Methods and Compositions for Ethanol Producing Cyanobacteria	Mar 2, 2011
Germany	69837531.9	Genetically Modified Cyanobacteria for the Production of Ethanol	Apr 11, 2007
Greece	3062508	Genetically Modified Cyanobacteria for the Production of Ethanol	Apr 11, 2007
Israel	198440	Closed Photobioreactor System for Production of Ethanol	Sep 28, 2013
Italy	27691BE/2007	Genetically Modified Cyanobacteria for the Production of Ethanol	Apr 11, 2007
Japan	5306823	Methods and Compositions for Ethanol Producing Cyanobacteria	Jul 5, 2013
Mexico	286222	Closed Photobioreactor System for Production of Ethanol	May 2, 2011
Mexico	290380	Methods and Compositions for Ethanol Producing Cyanobacteria	Sep 21, 2011
Mexico	309956	Genetically Modified Photoautotrophic Ethanol Producing Host cells, Method for Producing the Host Cells, Constructs for the transformation of the Host Cells, Method for Testing a Photoautotrophic Strain for a Desired Growth Property and Method of Producin	May 27, 2013
Netherlands	EP0963439	Genetically Modified Cyanobacteria for the Production of Ethanol	Apr 11, 2007

Portugal	EP0963439	Genetically Modified Cyanobacteria for the Production of Ethanol	Apr 11, 2007
Spain	2285759	Genetically Modified Cyanobacteria for the Production of Ethanol	Apr 11, 2007
Spain	2343776	Methods and Compositions for Ethanol Producing Cyanobacteria	Jun 13, 2011
Switzerland	EP0963439	Genetically Modified Cyanobacteria for the Production of Ethanol	Apr 11, 2007
United Kingdom	EP0963439	Genetically Modified Cyanobacteria for the Production of Ethanol	Apr 11, 2007
United Kingdom	1979481	Methods and Compositions for Ethanol Producing Cyanobacteria	Mar 2, 2011
United States of America	6,306,639	Genetically Modified Cyanobacteria for the Production of Ethanol, the Contructs and Method Thereof	Oct 23, 2001
United States of America	6,699,696	Genetically Modified Cyanobacteria for the Production of Ethanol, the Contructs and Method Thereof	Mar 2, 2004
United States of America	7,682,821	Closed Photobioreactor System for Continued Daily In Situ Production, Separation, Collection, and Removal of Ethanol From Genetically Enhanced Photosynthetic Organisms	Mar 23, 2010
United States of America	8,163,516	Selection of ADH in Genetically Modified Cyanobacteria for the Production of Ethanol	Apr 24, 2012
United States of America	8,323,958	Closed Photobioreactor System for Continued Daily In Situ Production of Ethanol From Genetically Enhanced Photosynthetic Organisms with Means for Separation and Removal of Ethanol	Dec 4, 2012
United States of America	8,372,613	Methods and Compositions for Ethanol Producing Cyanobacteria	Feb 12, 2013
United States of America	8,398,296	TRACK1-Magnetically Coupled System For Mixing	Mar 19, 2013
United States of America	8,404,466	TRACK1-Metabolically Enhanced Photoautotrophic Ethanol Producing Host Cells, Method for Producing the Host Cells, Constructs for the Transformation of the Host Cells, and Method of Producing Ethanol Using the Host Cells	Mar 26, 2013

United States of America	8,586,353	Closed Photobioreactor System for Continued Daily In Situ Production of Ethanol from Genetically Enhanced Photosynthetic Organisms with Means for Separation and Removal of Ethanol	Nov 19, 2013

10. Is this substance/product commercially available and if so, for how long has it been available on the commercial market?

Ethanol and other biofuels in general are commercially available. However, ethanol and other biofuels produced using algae are not commercially available.

a. If on the commercial market, are your competitors aware that the substance is commercially available in the U.S.?

Not applicable

b. If not already commercially available, describe what stage of research and development (R&D) the substance is in, and estimate how soon a market will be established.

Algenol's technology is in a continuous state of R&D. A commercial market will likely be established for biofuels from algae in late 2014 or early 2015; however, this is a speculative and there can be no assurances of the timing of the availability.

c. What is the substance used for and what type of product(s) does it appear in.

Transportation fuels. Other products can be made from ethanol, bio-crude, etc. and are too numerous to list, but those products are not the subject of the MCAN.

11. Describe whether a competitor could employ reverse engineering to identically recreate the substance?

Yes, the information is detailed enough that an industrial competitor could replicate Algenol's organism and production processes.

12. Do you assert that disclosure of this information you are claiming CBI would reveal:

a. Confidential processes used in manufacturing the substance.

RESPONSE: Yes.

b. If a mixture, the actual portions of the substance in the mixture.

RESPONSE: Not applicable.

c. Information unrelated to the effects of the substance on human health or the environment.

RESPONSE: Yes.

13. Provide the Chemical Abstract Service Registry Number for the product, if known. Is your company applying for a CAS number now or in the near future? If you have applied for a CAS number, include a copy of the contract with CAS.

We have not applied for a CAS number, nor do we expect to do so in the near future.

14. Is the substance or any information claimed CBI the subject of FIFRA regulation or reporting? If so, explain.

No.

C. Conclusion.

Algenol is start-up company pioneering a completely novel and proprietary technology, with first-of-its kind equipment and processing. To be responsive to the MCAN regulations, the Company has disclosed CBI relating to its entire process, including but not limited to:

- The identity of the cyanobacteria strain used in Algenol's technology and the genetic sequences and nature, materials, and methods of genetic enhancements relating to the cyanobacteria strain
- Algenol's processes for cultivating the cyanobacteria strain and controlling contamination
- Algenol's next-generation photobioreactor systems and processes for inoculating and cleaning photobioreactors
- Algenol's systems and processes for managing photobioreactor operations, including gas and liquid flow, and for mixing cultures of the cyanobacteria strain cultured in the photobioreactors
- Algenol's product extraction and recovery systems and processes.

Disclosure of the above information would reveal proprietary technology that Algenol has developed at substantial expense and that has not been publicly disclosed, thereby prejudicially conferring an advantage to Algenol's competitors and potentially barring the patentability of most if not all of the CBI.

For all for the reasons set forth above, there is ample support to substantiate the confidential nature of all of the information identified as CBI in Algenol's MCAN application. We trust that this letter provides you with the information you require in order to maintain the confidentiality of Algenol's CBI. If you have any further questions, please do not hesitate to contact me directly at (239) 498-2000.

Best regards,

Patrick Ahlm
Assistant Director, Government & Regulatory Affairs

4 Introduction

In accordance with 40 C.F.R. § 725.105, Algenol Biofuels Inc. (the Company or Submitter) is filing this consolidated Microbial Commercial Activity Notice (MCAN) with the United States Environmental Protection Agency (EPA) for the enhanced strains of cyanobacteria, or blue-green algae, (collectively, the Enhanced Algae) comprehensively detailed in following sections of this notice.

The Enhanced Algae are enhanced versions of [redacted] that will be used in the Company's Direct to Ethanol® technology for the production of biofuels, including ethanol, biodiesel, gasoline and jet fuel. The Enhanced Algae will be deployed in enclosed photobioreactors in order to convert carbon dioxide (CO_2) into ethanol. Waste algae from the ethanol production step provide the feedstock for a biomass-to-hydrocarbon fuels process. Hydrothermal liquefaction produces a crude bio-oil that is upgraded in a hydrotreater then distilled into the other aforementioned biofuels. Sunlight (photosynthesis), non-arable land and saltwater are the other principal requirements for the production of these advanced biofuels using the Company's technologies. In the course of the production processes all of the Enhanced Algae will be rendered biologically inactive and none will be intentionally released into the environment.

[redacted]

[redacted]

Generally speaking, cyanobacteria can be found in almost every terrestrial and aquatic habitat and perhaps innumerable species exist as they have adapted to their specific climates over time. Certain species of cyanobacteria, such as Spirulina, are sold as food and others are used in cosmetic products like face creams. The overwhelming majority of cyanobacterial species are innocuous. However, because a limited number of harmful toxic species do exist, Algenol has undertaken a robust and varied program of environmental studies and screening protocols to ensure that the Enhanced Algae do not pose an unreasonable risk to human health or the environment. These studies include full genetic sequencing and analyses to document the absence of the genetic sequences required to produce known toxins along with routine examination of working cultures to confirm that toxins are not produced. Gene transfer studies modeled on studies conducted by the National Institute of Health are also conducted, which document the new organisms' inability to transfer the inserted genes under conditions that would optimize such transfer. Perhaps most importantly, non-invasiveness studies using a wide variety of naturally occurring water samples were conducted that demonstrate the new organisms' inability to become invasive outside of the specific environment created inside the enclosed photobioreactor. These studies augment an initial laboratory-screening paradigm that ensures that none of the few known toxic species are utilized for any purpose. These studies, and a history of safe use, provide support for the conclusion that the new organisms are non-toxic, non-invasive and are not plant pest and, therefore, are safe to use for large-scale biofuel production.

The production of economically viable and environmentally friendly alternatives to petroleum-based fuels is important for a variety of significant reasons. The Company's technology offers a credible process for the creation of biofuels with productivity rates far exceeding first and second generation biofuels and production costs that are superior to traditional fuels. It is also important to note that the Company's technology offers a uniquely compelling process for the capture,

utilization and offset of vast amounts of CO_2 from anthropogenic sources while producing a valuable commodity. In consideration of these motivations and the demonstrable safety of the Enhanced Algae, the Company believes that commercialization of the technology without restriction should not only be approved, but also encouraged and supported, by EPA.

5 Submitter Information

Business Address:
Algenol Biofuels Inc.
16121 Lee Road
Fort Myers, FL 33912

Authorized Individual For Submission:
Patrick Ahlm
Assistant Director, Government and Regulatory Affairs
16121 Lee Road
Fort Myers, FL 33912
239.498.2000

Principle Technical Contacts:
Dr. Paul Roessler, Chief Scientific Officer
Dr. Benjamin McCool, Senior Vice President of Engineering
16121 Lee Road
Fort Myers, FL 33912
239.498.2000

6 Organism Identification Information

6.1 *Description of the Recipient Organism*

[redacted]

Figure 1 Redacted

Figure 2 Redacted

Figure 2: [redacted]

6.2 *Detailed Description of the Genetic Construction of the New Organisms*

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Algenol's ethanol-producing strains were genetically modified through the introduction of genes that enhance the production of ethanol. [redacted]

[redacted]

[redacted] Algenol's ethanol-producing strains will be produced using one of these approaches at commercial scale.

Figure 3 Redacted

Figure 3: Map of plasmid [redacted]

Figure 4 Redacted

Figure 4: Map of Plasmid [redacted]

Figure 5 Redacted

Figure 5: Map of Plasmid #1658

6.5 *References*:

Conway Y.A., Osman T., Konnan J.I., Hoffmann E.M., Ingram L.O. (1987)Promoter and nucleotide sequences of the *Zymomonas mobilis* pyruvate decarboxylase *J. Bacteriol*., 169: 949-54.

Mora I., Rascio N., La Rocca N. Di Bella, Monica; Andreoli, C. (2007) *Cyanobacterium aponinum*, a new Cyanoprokaryote from the micromat of Euganean thermal springs (Padua, Italy), *Algological Studies* 123:1-15.

Vidal R., López-Maury L., Guerrero M.G., Florencio F.J. (2009) Characterization of an alcohol dehydrogenase from the cyanobacterium *Synechocystis* sp. strain PCC 6803 that responds to environmental stress conditions via the Hik34-Rre1 two-component system. *J. Bacteriol*. 191:4383-91.

Krinke L, Wulff DL. (1987) OOP RNA, produced from multicopy plasmids, inhibits lambda cII gene expression through an RNase III-dependent mechanism. *Genes Dev.* 1:1005-13.

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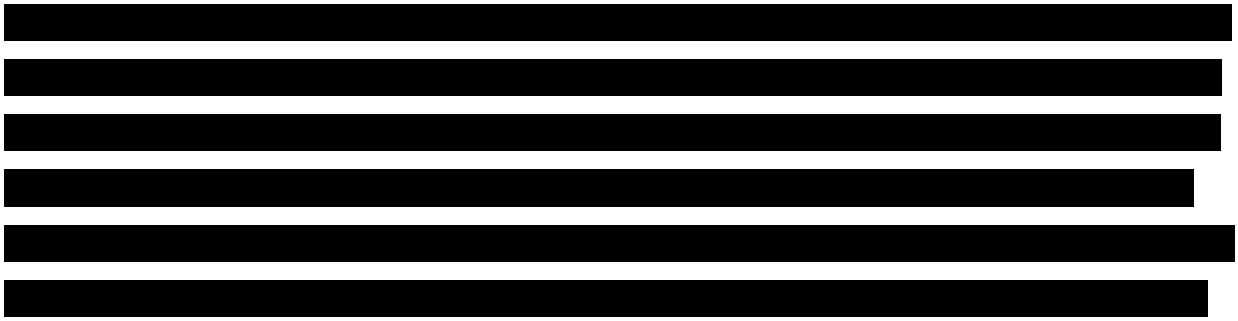
7 Manufacturing and Use

The simple process flow diagram below depicts the three stages of Direct to Ethanol® technology: (i) upstream processes necessary to prepare and deliver the principal inputs of enhanced algae, salt water and CO_2; (ii) photosynthetically driven cultivation, product creation and delivery of an ethanol rich biomass stream; and (iii) downstream processing to purify ethanol to fuel grade and convert the biomass to distillate range hydrocarbon fuels. Each of these stages is described in detail below. The processes depicted and described below work in concert to produce ethanol and remove it from the biomass that is then converted into other biofuels, all of the algae are thusly destroyed and process water is treated and recycled. There are no waste streams.



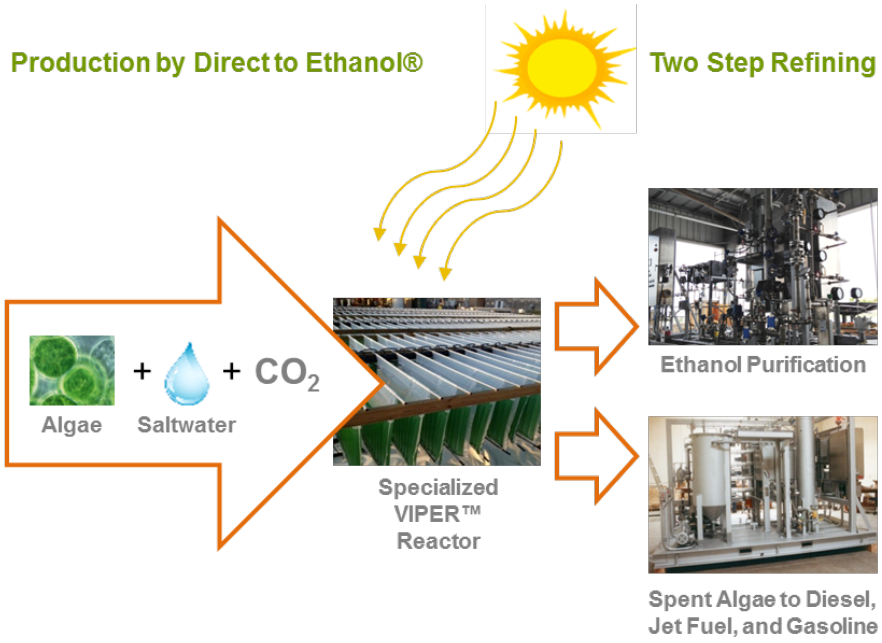


Figure 6: Simple Process Flow Diagram

7.1 *Upstream Processes*

7.1.1 Salt Water

[redacted]

7.1.2 Carbon Dioxide Feedstock

[redacted]

[redacted]

CO_2 is commercially available for enhanced oil recovery (EOR) for $15 to $35 per tonne. In 2008, in the U.S. more than 44 million tonnes of CO_2 was used for EOR. We expect that in the future industrial emitters, such as electric power plants, petroleum refineries, chemical plants, fertilizer manufacturers, and cement factories, will be able to capture CO_2 of the requisite purity and pressure for a lower cost than CO_2 for EOR. Low concentration flue gas CO_2 directly from a natural gas fired power plant is also a viable option. [redacted]

7.1.3 Nutrients for Enhanced Production

Adequate nutrient supply plays a critical role in algal growth. It directly affects the length of algal life cycle, biomass, ethanol and oxygen production rate, as well as carbon dioxide (CO_2) assimilation rate. The production of many cellular components relies on elements other than the carbon, hydrogen and oxygen supplied by photosynthetic reactions. Synthesis of amino and nucleic acids, lipids, photosynthetic pigments and a host of other compounds require additional elements. Primary nutrients are nitrogen and phosphorous as well as trace metals in very low concentrations. [redacted]

[redacted]

[redacted]

[redacted]

There are additional requirements for metals that are supplied at much lower concentrations and thus referred to as micronutrients. These compounds include but may not be limited to iron, zinc, copper, cobalt, manganese, magnesium, molybdate, and borate and are often accompanied by chelators. All of these compounds are dependent on the source water levels that may prove to be sufficient to fulfill the nutritional requirements of the cyanobacteria.

7.1.4 Cultivation and Fuel Production Process

Figure 7: VIPER™ photobioreactor systems, or Vertical Ethanol Greenhouses, deployed at Commercial Development Campus in Fort Myers, FL.

[redacted]

[redacted]

In general, the enclosure of a photobioreactor is transparent or translucent and will be constructed from flexible film materials, such as polyethylene, polypropylene, nylon and polyvinyl chloride, or rigid materials such as polycarbonate and polymethyl methacrylate, with openings formed in the enclosure. [redacted].

[redacted]

The photobioreactors are filled or nearly filled with liquid culture that comprises commerciallyavailable growth media and the Enhanced Algae that consume nutrients, CO_2 and energy from sunlight and secrete ethanol into the liquid culture.

The working cultures of Enhanced Algae in the photobioreactors make oxygen through photosynthesis, with the oxygen released into the liquid culture. Accumulation of excessive oxygen in the liquid culture inhibits ethanol production. [redacted]

[redacted]

7.2 *Two-Step Downstream Refining Process*

Algenol produces ethanol [redacted]. In addition to ethanol, algal biomass grows throughout the span of an ethanol production run. At the end of the run, spent algal biomass is separated from the ethanol-containing liquid medium. Then our two-step refining process is used to purify ethanol to fuel grade from the liquid medium and convert spent algal biomass to bio-crude to distillate range hydrocarbon fuels.

7.2.1 Ethanol Purification

The cornerstone enabling technology in our ethanol purification process is the patented vapor compression steam stripper or VCSS. The VCSS allows Algenol to purify the dilute ethanol to fuel grade with energy demand many times lower than conventional ethanol purification technologies. Most commercial ethanol facilities use distillation and molecular sieve

dehydration to concentrate ethanol from around 10% to fuel grade at or greater than 99.5% purity.

[redacted]

Figure 8 Redacted

Figure 8: [redacted]

Figure 9 Redacted

Figure 9: [redacted]

7.2.2 Hydrothermal Liquefaction Technology

Waste algal biomass from ethanol production will provide the feedstock for the biomass-to-hydrocarbon fuels process. [redacted] The output from the HTL unit will be crude bio-oil, an aqueous stream with dissolved organics and nutrients, and insoluble solids. The HTL bio-oil can upgraded in a hydrotreater unit to a hydrocarbon product that essential contains a mixture of liquid hydrocarbons in the range of diesel, jet and gasoline fuels. The upgraded product contains none of the oxygen, nitrogen or sulfur present in the crude bio-oil from HTL. The upgraded product can then be distilled into diesel, jet fuel and gasoline fractions.

The aqueous organic-rich effluent stream from HTL can then be sent to a catalytic hydrothermal gasification (CHG) unit to produce a mixed gas stream of methane and CO_2 and a stable aqueous stream containing dissolved nutrients. The water and nutrients will be recycled for biomass growth. The methane in the mixed gas stream can be used to generate electricity and heat in a combined heat and power unit (CHP), or processed to remove the carbon dioxide and give pure methane for sale as renewable natural gas. The CO_2 will be recycled for biomass growth. The CHG process also produces a small amount of insoluble solids containing phosphorus, and these solids will be used as nutrient recycle. [redacted]

Figure 10 Redacted

Figure 10: [redacted]

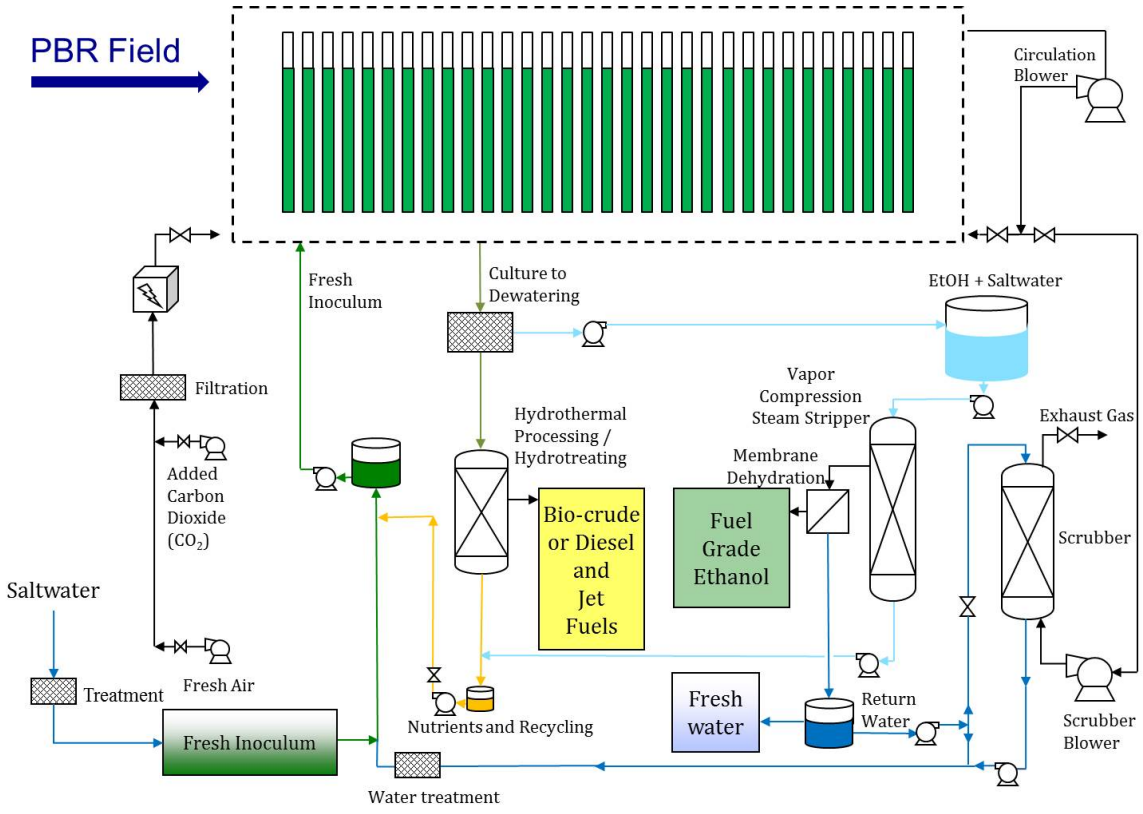


Figure 11: Detailed process flow diagram.

7.3 *Worker Exposure and Environmental Release*

Algenol has established a multi-faceted approach to establish safe working conditions and standard operating procedures to limit worker exposure to potentially hazardous materials and avoid unintentional environmental release of Enhanced Algae. A national Environmental Health and Safety (EH&S) consulting firm, Vanguard Environmental, has been engaged to conduct routine EH&S audits and to conduct annual safety training that is required for all staff. Additionally, the Company has established an EH&S committee that includes executive leadership and representatives from each of the Company's departments. Procedures for scale-up, culture transfer, culture maintenance and inoculation preparation are specifically designed

and strictly followed to limit employee exposure to, and environmental release of, Enhanced Algae and ensure quality inoculum is available for deployment. Cultures are contained in a variety of different enclosed vessels at all times, aseptic and sterile handling procedures are necessary because the Enhanced Algae are vulnerable to contaminants and incapable of thriving in natural environments. Scale-up logs are maintained to ensure standard operating procedures are followed and only appropriate inoculum is deployed.

Good laboratory practices have been instituted that require all personnel handling the Enhanced Algae to be thoroughly acquainted with the limited potential hazards of the utilized reagents, samples and solvents before commencing scale-up and inoculation procedures. Appropriate personal protective gear, including safety glasses, protective gloves and lab coats, are worn at all times. General laboratory safety training for handling potential biohazards is routinely conducted, as is equipment specific training that includes proper operating procedures for laminar flow hoods, autoclaves, ozone generators and sterile tube fusers, among other things. All personnel conducting scale-up, inoculation and operating procedures are qualified and skilled in microbiological techniques, especially sterile handling procedures.

7.4 *Scale-up Procedures*

[redacted]

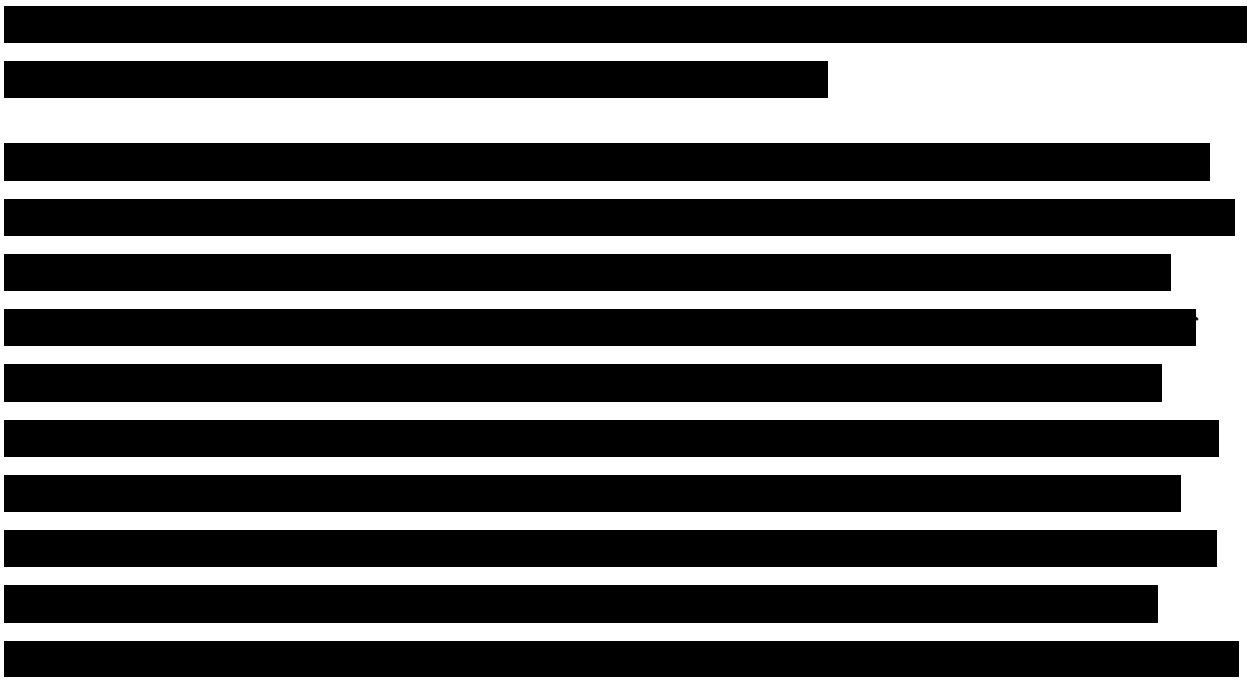


Figure 12 Redacted

Figure 12: Example of agar plate cultures. On agar plate lines, cultures are streaked the same way at each transfer to attain a relatively consistent amount of biomass on each plate. Note that each culture is labeled with information included in the scale-up log.

Figure 13 Redacted

Figure 13: Example of flask cultures. Flask cultures are established by transferring biomass from agar plates into 100mL of repression medium. Note that each culture is labeled with information included in the scale-up log.

[1] Repression media are the nutrient/trace metal mixture that maintain the cells in a repressed (i.e. non-ethanol producing) state. When the inoculum is transferred to the photobioreactors the media mixture changes to induction media, allowing the promoter to turn on and the gene for ethanol production to express. The Enhanced Algae include a nir-A promoter, which induces in the presence of nitrate. The repression media use urea instead of nitrate as a nitrogen source.

[redacted]

Figure 14 Redacted

Figure 14: Example of [redacted]

[redacted]

Figure 15 Redacted

Figure 15: Example of [redacted]

7.5 *Inoculation*

[redacted]

[redacted]

8 Human Health and Environmental Safety

The ability of Algenol's Direct to Ethanol® technology to produce transportation fuels by utilizing the Enhanced Algae in concert with sunshine, saltwater and marginal land will yield significant environmental benefits when deployed at commercial scale. Algenol's commitment to environmental safety starts at the very beginning of identifying and developing a candidate strain of algae in the laboratory. Candidate strains are rigorously screened not only for their performance characteristics with respect to, among other things, temperature, salinity and ethanol production in the specific conditions created inside the photobioreactor, but also for environmental considerations to ensure they are non-toxic, non-invasive and are not plant pests. The Company's proprietary screening paradigm is augmented by a battery of environmental studies that each host organism is subjected to at the appropriate stage of development. Along with a history of safe use, these studies have repeatedly yielded significantly similar results that demonstrate the Enhanced Algae's environmental and human health safety. The environmental studies include:

- Non-invasiveness testing to determine the Enhanced Algae's ability to thrive in the event of a spill onto soil or a variety of natural water samples;
- Whole genome sequence analysis and annotation in order to perform genome-wide screening for cyanotoxin pathway genes;
- Qualitative analysis of working cultures, both biomass and media, for the presence of cyanotoxins using high performance liquid chromatography; and
- Assessment of horizontal gene transfer potential.

A version of each of these studies relative to the Enhanced Algae are summarized below and full copies of each are included herewith as attachments. It is important to note that these studies have been designed with input from the Florida Department of Agriculture and Consumer Services in order to receive necessary state level authorizations for outdoor deployment of the Enhanced Algae at the Company's research and development campus and the potential commercial locations. These studies, first suggested and designed by Algenol's internal experts have evolved over time with input from the Florida Department and its experts. In many cases, the studies were made more stringent in order to observe outcomes in simulated extreme conditions. For example, the inoculation density of the non-invasiveness studies replicates a massive spill into a small body of water.

The studies attached herewith relate to the Enhanced Algae, but it is important to note that several other strains of similarly enhanced algae have been subjected to these environmental studies as they advanced to the outdoor research and development stage. Like the Enhanced Algae, the other strains were similarly incapable of thriving in natural water samples, lacked the ability to produce toxins and did not transfer the ethanologenic gene cassette under conditions optimal for such a transfer. The results of these studies considered in totality, strongly support the conclusion that the basic genetic enhancement of overexpressing fermentation pathway enzymes in cyanobacteria consistent with the Company's practices does not produce an unacceptable risk to the environment, especially when considered in light of the potential benefits of the technology.

8.1 *Environmental Studies*

[redacted]

[redacted]

[2] See Attachment 1.

[redacted]

8.2 *Confirmation of Non-Toxicity*[4]

[redacted]

8.3 *Assessment of Gene Transfer Potential*[5]

[redacted]

[3] See Attachment 2.
[4] See Attachment 3.
[5] See Attachment 4.

[redacted]

8.4 *Human Health Assessment*[6]

[redacted]

9 Current and Future Sites

9.1 *Current Site*

Algenol's current site, hereafter referred to as the integrated biorefinery, or IBR, is a collaborative effort that is funded in part by a $25 million grant from the American Recovery and

[6] See Attachment 5.

Reinvestment Act and administered by the DOE's Office of Energy Efficiency and Renewable Energy (EERE), Biomass Program. The National Renewable Energy Laboratory, The Georgia Institute of Technology, Membran Technology & Research, Inc., Florida Gulf Coast University and other public and private entities are also collaborating with Algenol on different aspects of commercializing Direct to Ethanol® technology, respectively. The primary objectives for the IBR include demonstrating the commercial potential of the technology, creating and saving hundreds of well-paying jobs and assessing the economic and environmental impact of a breakthrough novel technology. These objectives are consistent with the mission of DOE's Office of Energy Efficiency and Renewable Energy to invest in clean energy technologies that strengthen the economy, protect the environment and reduce dependence on foreign oil.

The IBR is a licensed zero discharge aquaculture facility operated in accordance with the Florida Aquaculture Best Management Practices (BMPs) and is situated on a 36-acre site adjacent to the Company's research and development facilities. At full capacity, it may consist of approximately 17 acres of plastic, fully enclosed and sealed photobioreactors with the remaining acreage utilized for supporting infrastructure, a processing pavilion, a greenhouse and a work areas including an office and laboratory building. To execute the project [redacted] Algenol has over 150 employees, including more than 50 doctorate and post-doctorate level professionals with a range of disciplines including environmental health and safety, toxicology, molecular biology, marine biology, genetics, analytical chemistry and bioinformatics.

Figure 16: Commercial Development Campus and IBR in Fort Myers, Florida

9.1.1 *Facility Description*

The physical address for the IBR is 16161 Lee Road Ft. Myers, FL 33912. The site is located in southeast Fort Myers, approximately 10 miles south of the City of Fort Myer's downtown area and approximately three miles southwest of the Southwest Florida International Airport. The site consists of approximately 36 acres of land north of Alico Road, approximately 1.1 miles northwest of the intersection of Alico Road and Interstate 75. The property is fairly isolated from the general public as it is zoned for heavy industrial use and for chemical and allied products and manufacturing use.

[redacted]

[redacted] The facility is equipped with a sophisticated security system and an armed security professional guards the site outside of normal business hours.

[redacted]

On December 28, 2010, DOE issued a Finding of No Significant Impact for the IBR that stated, among other things, that the funding of the IBR is consistent with DOE's goals under the objectives outlined in The Energy Independence and Security Act of 2007 and the American Recovery and Reinvestment Act of 2009. With respect to environmental resources, DOE

determined that for the IBR site, there would be no adverse impacts or that the impacts would be small, temporary, or both for all resources.

9.2 *Future Sites*

[redacted].

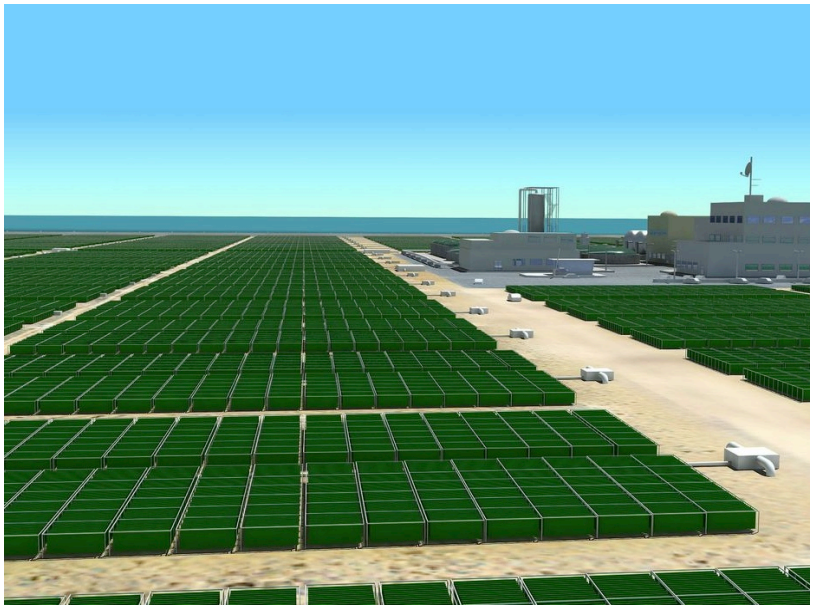


Figure 17: Artist rendering of future commercial facility.

10 Amount of Enhanced Algae to be Cultivated

[redacted]

11 Conclusion

Algenol's Direct to Ethanol® technology offers a compelling value propositions from an environmental perspective. The environmental studies included herewith demonstrate that the Enhanced Algae are non-toxic, non-invasive, are not plant pests and do not pose a threat to human health. Moreover, the technology has the ability to capture, re-use and offset CO_2, emissions and does not require freshwater or valuable farmland in order to domestically produce valuable transportation fuels. Accordingly, EPA should approve commercial scale deployment of the Enhanced Algae.

Attachment 1

Non-Invasiveness of the Ethanologenic Cyanobacterium Strain [redacted]

in Southwest Florida Environmental Waters

REDACTED

Attachment 2

Lack of Survival of the Cyanobacterium Strain [redacted] upon Spillage onto Dry Soil

REDACTED

Attachment 3

Screening of Cyanotoxin Biosynthesis Genes in Candidate Strains

REDACTED

Attachment 4

Assessment of the Horizontal Gene Transfer Potential of [redacted] plasmid from [redacted] to *Escherichia coli*

REDACTED

Attachment 5

Facts About Cyanobacteria & Cyanobacterial Harmful Algal Blooms

Department of Health and Human Services
Centers for Disease Control and Prevention
National Center for Environmental Health
Division of Environmental Hazards & Health Effects

(attached as a separate file)

Attachment 6

GenBank File with Genome Sequencing of [redacted]

(attached as a separate file - Redacted)

Facts About
Cyanobacteria &
Cyanobacterial Harmful Algal Blooms

Department of Health and Human Services
Centers for Disease Control and Prevention
National Center for Environmental Health
Division of Environmental Hazards & Health Effects

Facts about Cyanobacteria (Blue-green Algae) and Cyanobacterial Harmful Algal Blooms (CyanoHABs)

Cyanobacteria (blue-green algae)

Cyanobacteria are bacteria that grow in water and are photosynthetic (use sunlight to create food and support life). Cyanobacteria live in terrestrial, fresh, brackish, or marine water. They usually are too small to be seen, but sometimes can form visible colonies. Cyanobacteria have been found among the oldest fossils on earth and are one of the largest groups of bacteria. Cyanobacteria have been linked to human and animal illnesses around the world, including North and South America, Africa, Australia, Europe, Scandinavia, and China.

Cyanobacterial blooms and how they form

Cyanobacterial blooms occur when algae that are normally present grow exuberantly. Within a few days, a bloom can cause clear water to become cloudy. The blooms usually float to the surface and can be many inches thick, especially near the shoreline. Cyanobacterial blooms can form in warm, slow-moving waters that are rich in nutrients such as fertilizer runoff or septic tank overflows. Blooms can occur at any time, but most often occur in late summer or early fall.

They can occur in marine, estuarine, and fresh waters, but the blooms of greatest concern are the ones that occur in fresh water, such as drinking water reservoirs or recreational waters.

What a cyanobacterial bloom looks like

Some cyanobacterial blooms can look like foam, scum, or mats on the surface of fresh water lakes and ponds. The blooms can be blue, bright green, brown, or red and may look like paint floating on the water. Some blooms may not affect the appearance of the water. As algae in a cyanobacterial bloom die, the water may smell bad.

Cyanobacterial harmful algal blooms (CyanoHABs)

CyanoHABs are algae blooms that threaten people, animals, or the environment. They are dangerous for many reasons:

- Dense CyanoHABs can block sunlight and use up all the oxygen in the water, killing other plants and animals.
- Some cyanobacteria that can form CyanoHABs produce toxins that are among the most powerful natural poisons known. These toxins have no known antidotes.
- CyanoHABs can make people, their pets, and other animals sick. Often, the first sign that an HAB exists is a sick dog that has been swimming in an algae-filled pond.
- Children are at higher risk than adults for illness from CyanoHABs because they weigh less and can get a relatively larger dose of toxin.

Other effects of fresh-water CyanoHABs

- CyanoHABs can make drinking water smell and taste bad.
- They can make recreational areas unpleasant.

Species of cyanobacteria that form CyanoHABs in fresh water

- *Microcystis aeruginosa*
- *Anabaena circinalis*
- *Anabaena flos-aquae*
- *Aphanizomenon flos-aquae*
- *Cylindrospermopsis raciborskii*

Cyanotoxins

Cyanotoxins are a diverse group of chemical substances that are categorized by their specific toxic effects as follows:

- Neurotoxins affect the nervous system.
  - Anatoxin-a
  - Anatoxin-a(s)
  - Saxitoxin
  - Neosaxitoxin
- Hepatotoxins affect the liver.
  - Microcystins
  - Nodularins
  - Cylindrospermopsin
- Tumor promoters are chemicals that can increase tumor growth.
  - Microcystins
- Lipopolysaccharides are chemicals that can affect the gastrointestinal system.

See the table below for a list of cyanotoxins and their specific toxic mechanisms, their effects, the symptoms they cause, and treatments for poisoning.

How you could be exposed to CyanoHABs and cyanotoxins

- Drinking water that comes from a lake or reservoir with a CyanoHAB.
- Drinking untreated water.
- Engaging in recreational activities in waters with CyanoHABs.
- Inhaling aerosols from water-related activities such as jet-skiing or boating.
- Inhaling aerosols when watering lawns, irrigating golf-courses, etc. with pond water.
- Using cyanobacteria-based dietary supplements that are contaminated with microcystins.
- Receiving dialysis (this has been documented only in Brazil).

Types of illnesses people and animals can get from exposure to CyanoHABs

- ***Getting it on the skin*** may give people a rash, hives, or skin blisters (especially on the lips and under swimsuits).
- ***Inhaling water droplets*** from irrigation or water-related recreational activities can cause runny eyes and nose, a sore throat, asthma-like symptoms, or allergic reactions.
- ***Swallowing water*** that has cyanobacterial toxins in it can cause
  - Acute, severe gastroenteritis (including diarrhea and vomiting).
  - Liver toxicity (i.e., increased serum levels of liver enzymes). Symptoms of liver poisoning may takes hours or days to show up in people or animals. Symptoms include abdominal pain, diarrhea, and vomiting.
  - Kidney toxicity.
  - Neurotoxicity. These symptoms can appear within 15 to 20 minutes after exposure. In dogs, the neurotoxins can cause salivation and other neurologic symptoms, including weakness, staggering, difficulty breathing, convulsions, and death. People may have numb lips, tingling fingers and toes, or they may feel dizzy.

Testing for cyanobacterial toxins

- Most of the toxins require specialized testing that can take weeks.
- Some kits are available to test for microcystins on site.

How to protect yourself, your family, and your pets from exposure to CyanoHABs

- Don't swim, water ski, or boat in areas where the water is discolored or where you see foam, scum, or mats of algae on the water.
- If you do swim in water that might have a CyanoHAB, rinse off with fresh water as soon as possible.
- Don't let pets or livestock swim in or drink from areas where the water is discolored or where you see foam, scum, or mats of algae on the water.
- If pets (especially dogs) swim in scummy water, rinse them off immediately—do not let them lick the algae (and toxins) off their fur.
- Don't irrigate lawns or golf courses with pond water that looks scummy or smells bad.
- Report any "musty" smell or taste in your drinking water to your local water utility.
- Respect any water-body closures announced by local public health authorities.

How to treat people or animals that have been exposed to cyanobacterial toxins

- Get medical treatment right away if you think you, your pet, or your livestock might have been poisoned by cyanobacterial toxins.
- Remove people from exposure and give them supportive treatment.

How to help reduce the occurrence of CyanoHABs

- Reduce nutrient loading of local ponds and lakes by using only the recommended amounts of fertilizers and pesticides on your yard.
- Properly maintain your household septic system.
- Maintain a buffer of natural vegetation around ponds and lakes to filter incoming water.

How to get more information about cyanobacteria:

Federal

Centers for Disease Control and Prevention (CDC)
Harmful Algal Blooms (HABs) site
http://www.cdc.gov/habs
This site defines HABs; describes CDC's HABs-related activities; and provides links to data, publications, and other HABs resources.

Cyanobacteria site
http://www.cdc.gov/hab/cyanobacteria/
This site defines cyanobacteria; describes CDC's cyanobacteria-related activities; and provides links to data, publications, and other cyanobacteria resources.

Environmental Protection Agency (EPA)
Drinking Water Contaminant Candidate List Site
http://www.epa.gov/safewater/ccl/cclfs.html
This site provides information about EPA's list of contaminants that are not regulated, occur in public water systems, and may require regulation under the Safe Drinking Water Act. Algae that can be harmful are on this list.

International

State of Queensland Australia
HAB site
http://www.nrm.qld.gov.au/water/blue_green/index.html
This site describes the state's plans and procedures for agency responding to HABs.

World Health Organization
Water Site
http://www.who.int/topics/water/en/
This site provides links to drinking and recreational water quality, including the impacts of cyanobacteria and cyanobacterial toxins.

States

North Carolina Department of Health and Human Services
Occupational and Environmental Epidemiology program, HABs Site
http://www.epi.state.nc.us/epi/hab/
This site gives an overview of North Carolina's HAB program and provides links to the state's HAB-related surveillance, research, and education activities.

Cyanobacterial toxins, effects, signs and symptoms of poisoning, and therapy

Toxin	Acute Effect	Signs and Symptoms	Therapy
Anatoxin-a	Neurotoxicity	*Humans:* not documented. *Animals:* progression of muscle fasciculations, decreased movement, abdominal breathing, cyanosis, convulsions, death. *Birds:* opisthotonos ("s"-shaped neck).	Supportive care. Respiratory support may allow time for detoxification and respiratory recovery.
Anatoxin-a (s)	Neurotoxicity	*Humans:* not documented. *Pigs:* hypersalivation, mucoid nasal discharge, tremors, fasciculations, ataxia, diarrhea, recumbency. *Ducks:* regurgitation, paresis, opisthotonos, clonic seizures. *Mice:* lacrimation, hypersalivation, urination, defecation, death from respiratory arrest. *Rats:* red-pigmented ears.	Supportive care.
Cylindrospermopsin	Hepatotoxicity, renal toxicity, chromosome breakage, aneuploidy	*Humans:* enlarged liver, malaise, anorexia, vomiting, headache. *Mice:* huddling, anorexia, slight diarrhea, gasping respiration.	Supportive care.
Microcystins	Hepatotoxicity	*Humans:* elevated gamma-glutamyl transpeptidase. *Humans, mice:* elevated alanine aminotransferase. *Rats:* embryo lethality, teratogenicity. *Mammals:* weakness, reluctance to move, anorexia, pallor of extremities and mucous membranes, mental derangement, survivors may be photosensitized.	*Humans:* powdered charcoal, supportive care. *Animals:* cholestyramine.
Nodularin	Hepatotoxicity	*Humans:* Skin and eye irritation from skin contact. *Experimental systems:* inhibition of protein phosphatases, tumor-promoter.	Supportive care.
Saxitoxin, neosaxitoxin	Neurotoxicity	*Humans:* paresthesia and numbness of lips and mouth within ½ to 3 hours after exposure, extending to face, neck, extremities; motor weakness; incoordin-ation; respiratory and muscular paralysis. *Animals:* incoordination, death by respiratory failure.	Activated charcoal, artificial respiration. Supportive care.

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A Sanitized version of the GenBank file is not available.

Please contact me with any questions.

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